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Effect of Aminoacetonitrile, Iproniazid and Semicarbazide on the Tensile Strength of Experimental Granulation Tissue

By

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Aminoacetonitrile and β-aminopropionitrile decrease the tensile strength of skin wounds (KALLIOMÄKI, YLI-POHJA & KULONEN 1957) and increase the fragility of embryonal chick tissues (LEVENE & GROSS 1959), which are among the symptoms of experimental lathyrism. It is further established that iproniazid and semicarbazide aggravate the lathyrism (ROY, LIPTON, STRONG & BIRD 1959; JUVA, MIKKONEN, TUOMINEN & KULONEN 1959) and even cause its symptoms alone (DASLER 1958; NEUMAN, MAXWELL & MCCOY 1959).

The purpose of the experiments reported here was to explore the effect of the substances mentioned on the tensile strength of granulation tissue by a method permitting simultaneous chemical analysis. LEVENE (1961) studied recently the effect of numerous substances on the solubility of collagen and on the fragility of chick embryos; it will be seen below that our results agree well with his.

Experimental

Animals. Altogether 421 rats of Wistar origin (weight 99 ± 20 g in the beginning of the experimental periods) were used. After implantation they were kept in steel wire cages, 2 in each, and allowed to eat the standard food *ad libitum*. They were weighed weekly, but the sexes were not recorded.

Implantation and measurement of the tensile strength. The method used has been described elsewhere (VILJANTO & KULONEN 1962). Both "Sponcal" tampons (produced by Svenska Cellulosa Ab., Sundsvall, Sverige) and "Visella" viscose cellulose sponges (20 × 10 × 10 mm., manufactured by Säteri Oy. Ltd., Valkeakoski, Finland) were used, but comparisons were made only between granulomata produced by the same materials. The tensile strength of the healing skin wound was also measured with a similar arrangement on two perpendicular strips (1 cm. broad).

Test substances. The feeding of the test substances began 7 days before the implantation, except when otherwise stated. Aminoacetonitrile hydrosulphate (Abbot Laboratories, Chicago, gift of Dr. A. van den Hooff) was dissolved in distilled water. The solution was mixed with the food and its amount adjusted so that the daily dosage was

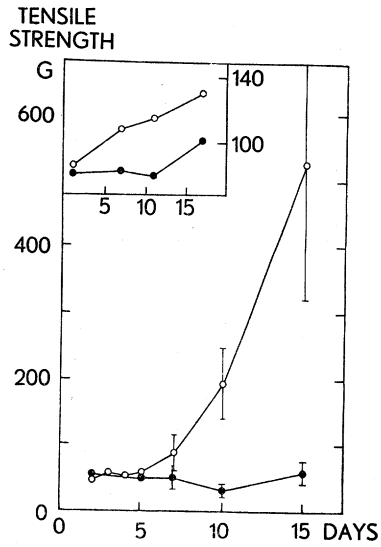

Fig. 1. Effect of administering aminoacetonitrile (● 25 mg/100 g daily ○ control) on the tensile strength of granulation tissue. Inserted is the average weight (in g) of the animals.

about 25, 50 or 100 mg/100 g body weight. Semicarbazide hydrochloride (E. Merck, Darmstadt) was also dissolved in distilled water and neutralized with sodium hydroxide. The solution was mixed with the food, and the rats received daily about 25 mg/100 g body weight. Iproniazid (1-*iso*nicotinyl-2-*iso*propylhydrazide) was used as "Marsilid" tablets (F. Hoffman-La Roche & Co. A.G., Basel), which were ground in a mortar, suspended in distilled water, shaken for at least 3 hours and neutralized with sodium hydroxide. The daily dosage was 2, 5 or 10 mg/100 g body weight.

Chemical analyses. Homogenized granulomata were combined to give samples containing 3–8 (in the average 5.3) sponge implants. The nitrogen was determined by micro-Kjeldahl combustion and subsequent distillation, the hydroxyproline by the method of Neuman & Logan (1950).

Results

Aminoacetonitrile. The dose of 100 mg/100 g/day was too toxic, and about half of the animals died from it shortly after implantation. At a dose of 25 mg/100 g/day none of the animals died, but even then the gain in body weight was appreciably reduced. Statistical treatment showed that at this dosage the decrease in tensile strength was significant (on 7th day $P < 0.05$, on 10th day $P < 0.001$), as indicated in fig. 1 (see also fig. 5 for hydroxyproline and fig. 6 for total nitrogen.)

Semicarbazide. A clear decrease (fig. 2) was observed both in weight gain and in tensile strength ($P < 0.01$ on 10th day).

Iproniazid. The preliminary experiment (fig. 3) indicated that iproniazid may enhance the tensile strength on the 3rd and 10th days ($P < 0.01$).

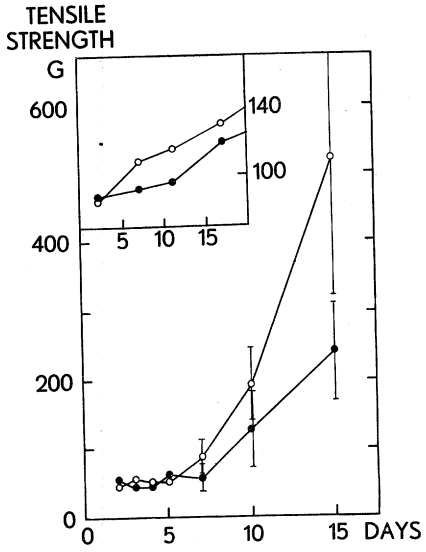

Fig. 2. Effect of administering semicarbazide (● 25 mg/100 g daily, ○ control) on the tensile strength of granulation tissue. Inserted is the average weight (in g) of the animals.

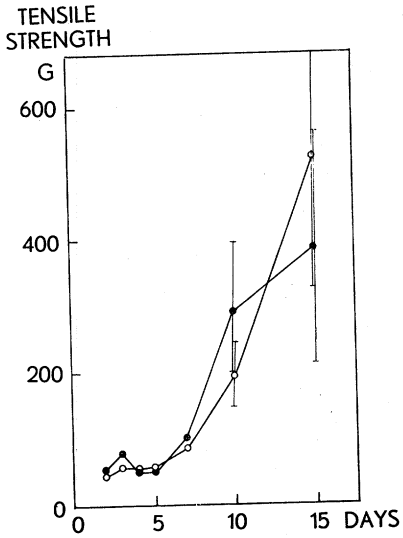

Fig. 3. Effect of administering iproniazid (● 5 mg/100 g daily, ○ control) on the tensile strength of granulation tissue.

Table 1.

The effect of various treatments with iproniazid on tensile strength in experimental granulomata in rats.

Iproniazid mg/day/rat	No. of rats	Beginning of treatment	Average weight in start	Weight gain g/day	Tensile strength g/sponge implant	
					3rd post-operational day	12th post-operational day
Control.....	27	–	116±16	2.26	44±12	465±95
2.....	21	7 days before operation	112±15	1.53	44±12	445±66
5.....	21	14 days before operation	111±12	1.42	35±12	429±70
5.....	37	at operation	116±16	0.50	31± 31)	459±93
5.....	21	7 days before operation	113±12	0.42	38± 8	439±67
10.....	21	7 days before operation	115± 9	0.21	32± 62)	414±97

The differences (*vs.* control) were statistically non-significant, except in groups marked 1) ($P < 0.02$) and 2) ($P < 0.01$).

This finding was later proved misleading, but it prompted a check with a series of 148 animals (Table I). It can be concluded that the effect on the tensile strength is always negative. It is not clear whether the effect of iproniazid is due to a non-specifically impaired general condition.

Effect of the weight of the rat. Twenty-eight rats were divided into three groups according to the weight: 101 ± 10 g, 167 ± 20 g and 318 ± 45 g (ages 45, 70 and 180 days, respectively). After implantation of the "Visella"-sponges, the tensile strength of implant tissue and skin wounds was measured. After three days there were no clear differences in granulomata, but the skin wounds were less strong ($P < 0.01$) in the group of smallest animals. At the 12th day the tensile strength of the skin wounds seemed to be almost proportional to the weight of the animals, but in the granulomata the differences were not statistically significant.

To get further evidence on this point, all the results from untreated animals were divided into seven groups according to the weight of the animals. The averages of the tensile strength were plotted against the weight (fig. 4). The strength of skin scars seems to increase continuously, presumably because of the thicker skin in older animals. The strength of the granulation tissue seems to depend on the weight of the animal up to a certain limit (about 140 g).

Chemical analyses. It is obvious that the amount of hydroxyproline (fig. 5) is correlated with the tensile strength (fig. 1) in normal implants. In samples from aminoacetonitrile-treated rats there was a discrepancy between the tensile strength and the synthesis of total hydroxyproline. In normal implants the nitrogen content rose steadily during the first 15 days, but in aminoacetonitrile-treated animals it persisted at 5th day level (fig. 6).

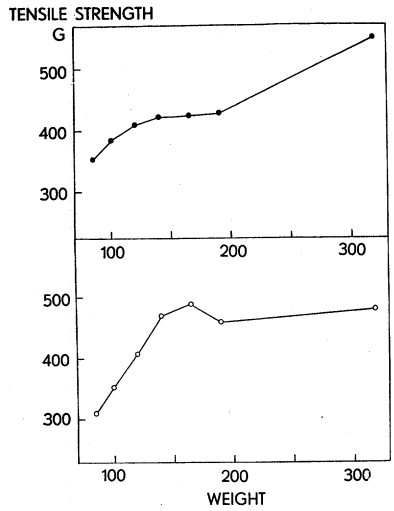

Fig. 4. Average tensile strength of granulation tissue (below) and of skin wound (top, 1 cm. broad strips) in untreated animals of different weights (12 days after operation).

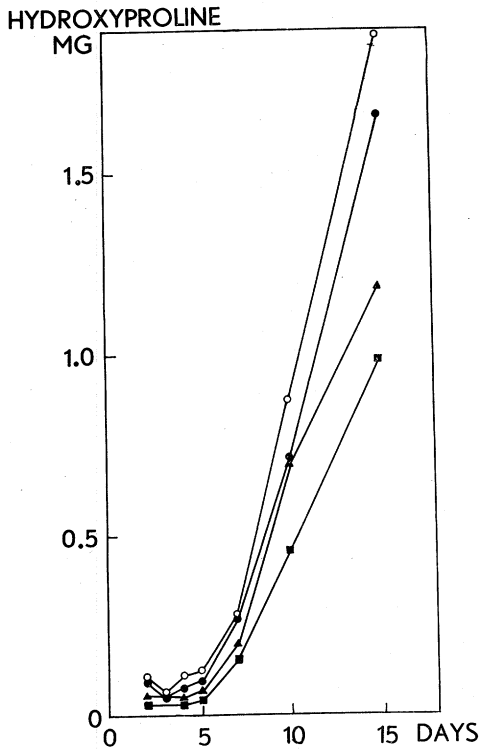

Fig. 5. Total hydroxyproline per sponge implant at different times after implantation (○ control, ● 5 mg/100 g iproniazid daily, ▲ semicarbazide 25 mg/100 g daily, ■ amino-acetonitrile 50 mg/100 g daily).

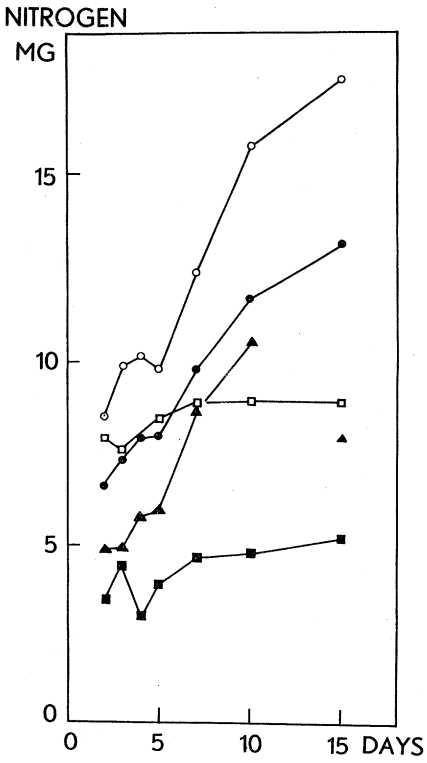

Fig. 6. Total nitrogen per sponge implant at different times after implantation (○ control, ● iproniazid 5 mg/100 g daily, ▲ semicarbazide 25 mg/100 g daily, □ aminoacetonitrile 25 mg/100 g daily, ■ aminoacetonitrile 50 mg/100 g daily.

Discussion

It was expected that aminoacetonitrile would cause a discrepancy between the amount of hydroxyproline and the tensile strength because of the increased solubility of collagen. It is obvious that there developed little tensile strength in aminoacetonitrile treated rats (fig. 1). The synthesis of hydroxyproline was retarded, but by no means completely inhibited (Fig. 5). This is in agreement with the concept that in lathyrism the soluble forms of collagen do not mature into mechanically strong fibres. The results for semicarbazide and iproniazid seem to lie between the control and that of aminoacetonitrile and may be partly non-specific.

Before the collagen synthesis begins in the sponge implants, there is a "lag phase". Already during that time the amount of nitrogen increased. During the first days there occurred a passive imbibition by the sponge with tissue fluids and the immigration of cells. The difference between various groups during these first days was not very large, but it is remarkable

that the amount of nitrogen did not increase in the aminoacetonitrile treated rats as in the other groups, and most in the control group. There seem no data available on the factors that regulate this protein accumulation before the collagen synthesis. If the nitrogen were derived solely from passive imbibition with tissue fluids, there would not be so much difference between the groups. Many explanations can be tentatively suggested for this effect of aminoacetonitrile: (1) impairment in the growth of capillaries, thus restricting the blood flow and metabolic development in general, (2) impairment of the division and proliferation of cells or (3) impairment of the synthesis of cellular proteins, with subsequent deficient maturation of the collagen-synthesizing systems. The incorporation of amino acids into collagen is decreased in lathyrism studied *in vivo* with guinea pigs (MARTIN, GROSS, PIEZ & LEWIS 1961). This could be explained by an effect on capillaries (which are weak in lathyrism), but the decreased collagen synthesis can be produced also in granuloma slices (KULONEN, SALMI & JUVA 1961). There is a general, but not understood, impairment of amino acid metabolism. The similar effect of the "carbonyl fixatives", semicarbazide and iproniazid, may be due to their action on general metabolism.

Up to a certain limit the growth of the granuloma depends on the nutritional stage of the animal (WILLIAMSON, MCCARTHY & FROMM 1951). In smaller animals the implant produces a larger strain on the animal, and the capillary growth also may be proportionally less.

Summary

Administration of either aminoacetonitrile or semicarbazide decreased the tensile strength in developing experimental granulation tissue. Similar, but probably less specific, effects were observed after administration of *iso*propyl*iso*nicotinic acid hydrazide.

The weight and the nutritional state of the animals were important factors in the production of tensile strength in healing wounds and in granulation tissue.

Normally the tensile strength in the control sponge implants increased in parallel with the amount of hydroxyproline in the granulation tissue. In aminoacetonitrile treated animals the tensile strength developed slowly, but the production of hydroxyproline was not inhibited to the same degree. The total nitrogen in the granulation tissue began to rise already before the collagen synthesis, and most rapidly in the control samples. In aminoacetonitrile-treated animals the nitrogen content of the granulation tissue persisted at the 5th day level.

Acknowledgements.

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